REPORT

Study Title

ASSESSMENT OF ACUTE ORAL TOXICITY WITH [redacted]

IN THE RAT (ACUTE TOXIC CLASS METHOD)

Author

[redacted]

Study completion date

04 April 2007

Test Facility

NOTOX B.V.
Hambakenwetering 7
5231 DD 's-Hertogenbosch
The Netherlands

Laboratory Project Identification

NOTOX Project [redacted]
NOTOX Substance [redacted]

1. CONTENTS

2. STATEMENT OF GLP COMPLIANCE

NOTOX B.V., 's-Hertogenbosch, The Netherlands

The study described in this report has been correctly reported and was conducted in compliance with:

The Organization for Economic Cooperation and Development (OECD) Good Laboratory Practice Guidelines (1997).

Which essentially conform to:

The United States Food and Drug Administration Good Laboratory Practice Regulations.

The United States Environmental Protection Agency Good Laboratory Practice Regulations.

The sponsor is responsible for Good Laboratory Practice (GLP) compliance for all test substance information unless determined by NOTOX.

Analysis of stability, homogeneity and concentration of the test substance under test conditions was not performed as part of this study.

NOTOX B.V.

[redacted]
Study Director

[redacted]
Section Head Toxicology

[redacted]

Date: 4 April 2007 Date: 05 april 2007

3. QUALITY ASSURANCE STATEMENT

NOTOX B.V., 's-Hertogenbosch, The Netherlands

This report was inspected by the NOTOX Quality Assurance Unit to confirm that the methods and results accurately and completely reflect the raw data.

The dates of Quality Assurance inspections are given below.
During the on-site process inspections procedures applicable to this type of study were inspected.

The reporting date is the date of reporting to the Study Director. The QAU report was then forwarded to the Test Facility Management.

Type of inspections	Phase/Process	Start Inspection date	End Inspection date	Reporting date
Study	Protocol	19-Oct-06	19-Oct-06	19-Oct-06
	Report	12-Jan-07	12-Jan-07	12-Jan-07
Process	**Test substance unit** Test substance handling	15-Aug-06	18-Aug-06	21-Aug-06
	SPF unit Test substance handling Exposure Observation/Measurement Specimen handling	07-Nov-06	13-Nov-06	21-Nov-06
	Pathology unit Observation/Measurement	23-Aug-06	30-Aug-06	07-Sep-06

Head of Quality Assurance
[redacted]

[redacted]

Date: 16/1/07

4. SUMMARY

Assessment of acute oral toxicity with [redacted] in the rat (Acute Toxic Class Method).

The study was carried out based on the guidelines described in:
OECD No.423 (2001) "Acute Toxicity-Oral, Acute Toxic Class Method"
EC, Council Directive 67/548/EEC, Annex V, B.1 tris (2004) "Acute Oral Toxicity"
EPA, OPPTS 870.1100 (2002), "Acute Oral Toxicity - Acute Toxic Class Method"
JMAFF guidelines (2000) including the most recent partial revisions.

Initially, [redacted] was administered by oral gavage to three female Wistar rats at 2000 mg/kg body weight. In a stepwise procedure additional groups of females were dosed at 300 mg/kg body weight. All animals were subjected to daily observations and weekly determination of body weight. Macroscopic examination was performed on the day of death or after terminal sacrifice (Day 15).

The incidence of mortality was as follows, presented in chronological order of treatment:

Dose level	Mortality
2000 mg/kg	3/3
300 mg/kg	1/3
300 mg/kg	0/3

The decedents were found between days 1 and 2 post-treatment.

Clinical signs observed during the study period were as follows:

Dose level	Clinical signs
2000 mg/kg	Lethargy, hunched posture, laboured respiration, piloerection and ptosis.
300 mg/kg	Lethargy, hunched posture, uncoordinated movements, piloerection and ptosis.

The surviving animals had recovered from the symptoms between Days 2 and 3.

The body weight gain shown by the animals over the study period was considered to be normal.

No abnormalities were found at macroscopic post mortem examination of the animals.

The oral LD50 value of [redacted] in Wistar rats was established to be within the range of 300-2000 mg/kg body weight.

According to the OECD 423 test guideline, the LD50 cut-off value was considered to be 500 mg/kg body weight.

Based on these results:
- according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) of the United Nations (New York and Geneva, 2003), [redacted] should be classified as: harmful if swallowed (Category 4) for acute toxicity by the oral route.
- according to the EC criteria for classification and labelling requirements for dangerous substances and preparations (Council Directive 67/548/EEC), [redacted] should be labelled as: harmful if swallowed (R22).

5. INTRODUCTION

5.1. Preface

Sponsor	[redacted]
Study Monitor	[redacted]
Test Facility	NOTOX B.V. Hambakenwetering 7 5231 DD 's-Hertogenbosch The Netherlands
Study Director	[redacted]
Study Plan (in-life phase)	Start : 26 October 2006 Completion : 16 November 2006

5.2. Aims of the study

The objective of this study was to assess the toxicity of the test substance when administered in a single dose to female rats at one or more defined dosages. Furthermore, the results of the study allowed the test substance to be ranked according to most classification systems, currently in use.
This study should provide a rational basis for risk assessment in man.
The oral route was selected as it is a possible route of human exposure during manufacture, handling or use of the test substance.

5.3. Guidelines

As required by the Dutch Act on Animal Experimentation (February 1997), the study protocol was reviewed and agreed by the Laboratory Animal Welfare Officer and the Ethical Committee of NOTOX (DEC NOTOX 03-42) The study procedures described in this report were based on the following guidelines:

Organisation for Economic Co-operation and Development (OECD), OECD Guidelines for Testing of Chemicals, Section 4, Health Effects. No.423, "Acute Oral Toxicity - Acute Toxic Class Method", 2001

European Community (EC), Council Directive 67/548/EEC, Annex V, Part B, Methods for the Determination of Toxicity, as last amended by Commission Directive 2004/73/EC, B.1 tris: "Acute Toxicity (Oral) - Acute Toxic Class Method", 2004.

United States Environmental Protection Agency (EPA). Health Effects Test Guidelines, OPPTS 870.1100, Acute Oral Toxicity. Office of Prevention, Pesticides and Toxic Substances (7101), EPA 712-C-98-190, 2002.

Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), 12 Nousan, Notification No 8147, November 2000, including the most recent partial revisions.

5.4. Storage and retention of records and materials

Records and materials pertaining to the study including protocol, raw data, specimens (except specimens requiring refrigeration or freezing) and the final report are retained in the NOTOX archives for a period of at least 10 years after finalization of the report. After this period, the sponsor will be contacted to determine whether raw data and specimens should be returned to them, retained or destroyed on their behalf.

Those specimens requiring refrigeration or freezing will be retained by NOTOX for as long as the quality of the specimens permits evaluation but no longer than three months after finalization of the report.

NOTOX will retain a test substance sample until the expiry date, but no longer than 10 years after finalization of the report. After this period the sample will be destroyed.

6. MATERIALS AND METHODS

6.1. Test substance

6.1.1. Test substance information

Identification	[redacted]
Structure	[redacted]
Molecular formula	[redacted]
Molecular weight	1317.45
Description	Dark purple powder
Batch	0619201
Purity	>95% (NMR)
Test substance storage	At room temperature in the dark
Stability under storage conditions	Stable
Expiry date	01 January 2008

6.1.2. Study specific test substance information

pH (1% in water, indicative range)	8.1 – 8.3 (determined at NOTOX)
Stability at higher temperatures	Maximum temperature 75°C, maximum duration 48 hours
Stability in vehicle:	
• Propylene glycol	Not indicated
Solubility in vehicle:	
• Propylene glycol	Not indicated

6.1.3. Test substance preparation

Vehicle	Propylene glycol (Merck, Darmstadt, Germany) (specific gravity 1.036)
Rationale	The vehicle was selected based on trial formulations performed at NOTOX and on test substance data supplied by the sponsor.
Preparation	The formulations (w/w) were prepared within 4 hours prior to dosing. Homogeneity was accomplished to a visually acceptable level. Adjustment was made for specific gravity of the vehicle. The concentration of the test substance in vehicle was varied to allow constant dosage volume in terms of ml/kg body weight.

6.2. Test System

Species	Rat, Wistar strain Crl:WI (outbred, SPF-Quality). Recognised by international guidelines as the recommended test system (e.g. OECD, EC). Source: Charles River Deutschland, Sulzfeld, Germany.
Number of animals	9 Females (nulliparous and non-pregnant). Each dose group consisted of 3 animals.
Age and body weight	Young adult animals (approx. 10-11 weeks old) were selected. Body weight variation did not exceed +/- 20% of the sex mean.
Identification	Earmark.
Health inspection	A health inspection was performed prior to commencement of treatment, to ensure that the animals were in a good state of health.

6.3. Animal husbandry

Conditions
Animals were housed in a controlled environment, in which optimal conditions were considered to be approximately 15 air changes per hour, a temperature of 21.0 ± 3.0°C (actual range: 21.4 – 23.1°C), a relative humidity of 30-70% (actual range: 40 - 69%) and 12 hours artificial fluorescent light and 12 hours darkness per day.

Accommodation
Group housing of 3 animals per cage in labelled Macrolon cages (MIV type; height 18 cm.) containing sterilised sawdust as bedding material (Litalabo, S.P.P.S., Argenteuil, France) and paper as cage-enrichment (Enviro-dri, Wm. Lillico & Son (Wonham Mill Ltd), Surrey, United Kingdom).
Acclimatisation period was at least 5 days before start of treatment under laboratory conditions.

Diet
Free access to pelleted rodent diet (SM R/M-Z from SSNIFF® Spezialdiäten GmbH, Soest, Germany).

Water
Free access to tap water.

Results of analysis for each batch of diet (nutrients) and results of quarterly analysis of diet (contaminants), sawdust, paper and water were assessed and did not reveal any findings that were considered to have affected the study integrity. All certificates and results of analysis are retained in the NOTOX archives.

6.4. Study design

The toxicity of the test substance was assessed by stepwise treatment of groups of 3 females. The first group was treated at a dose level of 2000 mg/kg. The absence or presence of mortality of animals dosed at one step determined the next step, based on the test procedure defined in the guidelines. The onset, duration and severity of the signs of toxicity were taken into account for determination of the time interval between the dose groups.

6.5. Treatment

Method	Oral gavage, using plastic feeding tubes.
Fasting	Food was withheld overnight (for a maximum of 20 hours) prior to dosing until 3-4 hours after administration of the test substance.
Frequency	Single dosage, on Day 1.
Dose level (volume)	2000 mg/kg (10 ml/kg) body weight. 300 mg/kg (10 ml/kg) body weight.

6.6. Observations

Mortality/Viability	Twice daily. The time of death was recorded as precisely as possible.
Body weights	Days 1 (pre-administration), 8 and 15 and at death (if found dead after Day 1).
Clinical signs	At periodic intervals on the day of dosing (Day 1) and once daily thereafter, until Day 15. The symptoms were graded according to fixed scales and the time of onset, degree and duration were recorded: Maximum grade 4: grading slight (1) to very severe (4) Maximum grade 3: grading slight (1) to severe (3) Maximum grade 1: presence is scored (1).
Necropsy	The animals surviving to the end of the observation period were sacrificed by oxygen/carbon dioxide procedure. All animals assigned to the study were subjected to necropsy and descriptions of all internal macroscopic abnormalities recorded.

6.7. Electronic data capture

Observations/measurements in the study were recorded electronically using the following programme(s):
REES version 1.5 (REES scientific, Trenton, NJ, USA): Environmental monitoring.
TOXDATA version 8.0 (NOTOX B.V., 's-Hertogenbosch, The Netherlands): Clinical signs, Body weights.

6.8. Interpretation

The oral LD50 value of the test substance was ranked within the following ranges: 0-5, 5-50, 50-300 or 300-2000 mg/kg b.w. or as exceeding 2000 mg/kg b.w. The LD50 cut-off value was established based on OECD guideline 423.
No statistical analysis was performed (The method used is not intended to allow the calculation of a precise LD_{50} value).

The results were evaluated according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) of the United Nations (New York and Geneva, 2003) and the EC criteria for classification and labelling of dangerous substances and preparations (Council Directive 67/548/EEC and all adaptations to technical progress and amendments of this Directive published in the Official Journal of the European Communities).

6.9. List of deviations

6.9.1. List of protocol deviations

There were no deviations from the protocol.

6.9.2. List of standard operating procedures deviations

Any deviations from standard operating procedures were evaluated and filed in the study file. There were no deviations from standard operating procedures that affected the integrity of the study.

7. RESULTS

7.1. Mortality (Table 1)

The incidence of mortality was as follows, presented in chronological order of treatment:

Dose level	Mortality	Date of treatment
2000 mg/kg	3/3	26 November 2006
300 mg/kg	1/3	31 November 2006
300 mg/kg	0/3	02 December 2006

The decedents were found between days 1 and 2 post-treatment (Table 1).

7.2. Clinical Signs (Table 2)

Clinical signs observed during the study period were as follows:

Dose level	Clinical signs
2000 mg/kg	Lethargy, hunched posture, laboured respiration, piloerection and ptosis.
300 mg/kg	Lethargy, hunched posture, uncoordinated movements, piloerection and ptosis.

The surviving animals had recovered from the symptoms between Days 2 and 3.

7.3. Body Weights (Table 3)

The body weight gain shown by the surviving animals over the study period was considered to be similar to that expected of normal untreated animals of the same age and strain.

7.4. Macroscopic Findings (Table 4)

No abnormalities were found at macroscopic post mortem examination of the animals.

8. CONCLUSION

The oral LD50 value of [redacted] in Wistar rats was established to be within the range of 300-2000 mg/kg body weight.

According to the OECD 423 test guideline, the LD50 cut-off value was considered to be 500 mg/kg body weight.

Based on these results:

- according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) of the United Nations (New York and Geneva, 2003), [redacted] should be classified as: harmful if swallowed (Category 4) for acute toxicity by the oral route.
- according to the EC criteria for classification and labelling requirements for dangerous substances and preparations (Council Directive 67/548/EEC), [redacted] should be labelled as: harmful if swallowed (R22).

TABLE 1 MORTALITY DATA

TEST DAY	1	1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
HOURS AFTER TREATMENT	0	2	4														
FEMALES 2000 MG/KG	-	-	1	2	-	-	-	-	-	-	-	-	-	-	-	-	-
FEMALES 300 MG/KG	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FEMALES 300 MG/KG	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

TABLE 2 CLINICAL SIGNS

TEST DAY		1	1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
HOURS AFTER TREATMENT	MAX GRADE	0	2	4														
FEMALES 2000 MG/KG																		
ANIMAL 1																		
Behavior																		
Lethargy	(3)	-	-	2	+													
Posture																		
Hunched posture	(1)	-	1	1	+													
Breathing																		
Laboured respiration	(3)	-	-	1	+													
Skin / fur / plumage																		
Piloerection	(1)	-	-	1	+													
Various																		
Ptosis	(3)	-	-	1	+													
ANIMAL 2																		
Behavior																		
Lethargy	(3)	-	-	2	+													
Posture																		
Hunched posture	(1)	-	1	1	+													
Breathing																		
Laboured respiration	(3)	-	-	1	+													
Skin / fur / plumage																		
Piloerection	(1)	-	-	1	+													
Various																		
Ptosis	(3)	-	-	1	+													
ANIMAL 3																		
Posture																		
Hunched posture	(1)	-	1	-	+													
FEMALES 300 MG/KG																		
ANIMAL 4																		
Posture																		
Hunched posture	(1)	-	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Gait / motility																		
Uncoordinated movements	(3)	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ANIMAL 5																		
Behavior																		
Lethargy	(3)	-	-	1	+													
Posture																		
Hunched posture	(1)	-	1	1	+													
Gait / motility																		
Uncoordinated movements	(3)	-	1	1	+													
Skin / fur / plumage																		
Piloerection	(1)	-	-	1	+													
Various																		
Ptosis	(3)	-	-	1	+													

- = SIGN NOT OBSERVED / . = OBSERVATION NOT PERFORMED / + = ANIMAL DEAD

TABLE 2 CLINICAL SIGNS

TEST DAY HOURS AFTER TREATMENT	MAX GRADE	1 0	1 2	1 4	2	3	4	5	6	7	8	9	10	11	12	13	14	15
FEMALES 300 MG/KG																		
ANIMAL 6																		
Posture																		
Hunched posture	(1)	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gait / motility																		
Uncoordinated movements	(3)	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FEMALES 300 MG/KG																		
ANIMAL 7																		
No clinical signs noted		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ANIMAL 8																		
No clinical signs noted		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ANIMAL 9																		
No clinical signs noted		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

- = SIGN NOT OBSERVED / . = OBSERVATION NOT PERFORMED / + = ANIMAL DEAD

TABLE 3 BODY WEIGHTS (GRAM)

SEX/DOSE LEVEL	ANIMAL	DAY 1	DAY 8	DAY 15
FEMALES 2000 MG/KG				
	1 *	205	---	---
	2 *	200	---	---
	3 **	249	---	---
	MEAN	218	---	---
	ST.DEV.	27	---	---
	N	3	0	0
FEMALES 300 MG/KG				
	4	220	239	252
	5 **	209	---	---
	6	195	213	228
	MEAN	208	226	240
	ST.DEV.	13	18	17
	N	3	2	2
FEMALES 300 MG/KG				
	7	239	275	292
	8	262	309	316
	9	241	281	296
	MEAN	247	288	301
	ST.DEV.	13	18	13
	N	3	3	3

* Found dead at day 2. Bodyweight at death: animal 1 = 192 g, animal 2 = 191 g
** Found dead at day 1.

TABLE 4 MACROSCOPIC FINDINGS

ANIMAL	ORGAN	FINDING	DAY OF DEATH
FEMALES 2000 MG/KG			
1		No findings noted	Spontaneous death Day 2 after treatment
2		No findings noted	Spontaneous death Day 2 after treatment
3		No findings noted	Spontaneous death Day 1 after treatment
FEMALES 300 MG/KG			
4		No findings noted	Scheduled necropsy Day 15 after treatment
5		No findings noted	Spontaneous death Day 1 after treatment
6		No findings noted	Scheduled necropsy Day 15 after treatment
FEMALES 300 MG/KG			
7		No findings noted	Scheduled necropsy Day 15 after treatment
8		No findings noted	Scheduled necropsy Day 15 after treatment
9		No findings noted	Scheduled necropsy Day 15 after treatment